DEVELOPMENTAL MORPHOLOGY OF ASCOMYCETES

VI. THERMOASCUS AURANTIACUS

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This paper is the sixth in a series on the developmental morphology of the Ascomycetes and deals with *Thermoascus aurantiacus* Miehe. The culture used in the present study is not the type, and it was isolated from saw dust from Parana pine; it was received from the Commonwealth Mycological Institute, Kew, England, under the No IMI 67936. This was one of the cultures studied by APINIS (1967) along with culture No. IMI 91787 which was designated by him as the neotype.

The fungus was grown on YpSs agar medium and the various stages of the development of the fruit bodies were studied by making tease mounts stained with 0.1% lactofuchsin as recommended by CARMICHAEL (1955) and sectioning the materials by the paraffin method as described by JOHANSEN (1940) and PURVIS, COLLIER and WALLS (1964). Tease mounts were also used in the study of the anamorph. For the study of the germination of ascospores methods described earlier (SUBRAMANIAN and RAJENDRAN, in press) were followed.

DESCRIPTION OF THE FUNGUS

Colonies on YpSs agar medium growing rapidly at a temperature of 38-40°C, attaining a diameter of 7-8 cm in two or three days, white at first, later becoming light grey-buff. Surface growth mucoraceous, becoming granular and pale yellow, then to orange buff, bright brick-red; and finally almost brown in age (within the next two or three days). Exudate present on the surface of the culture as shining droplets.

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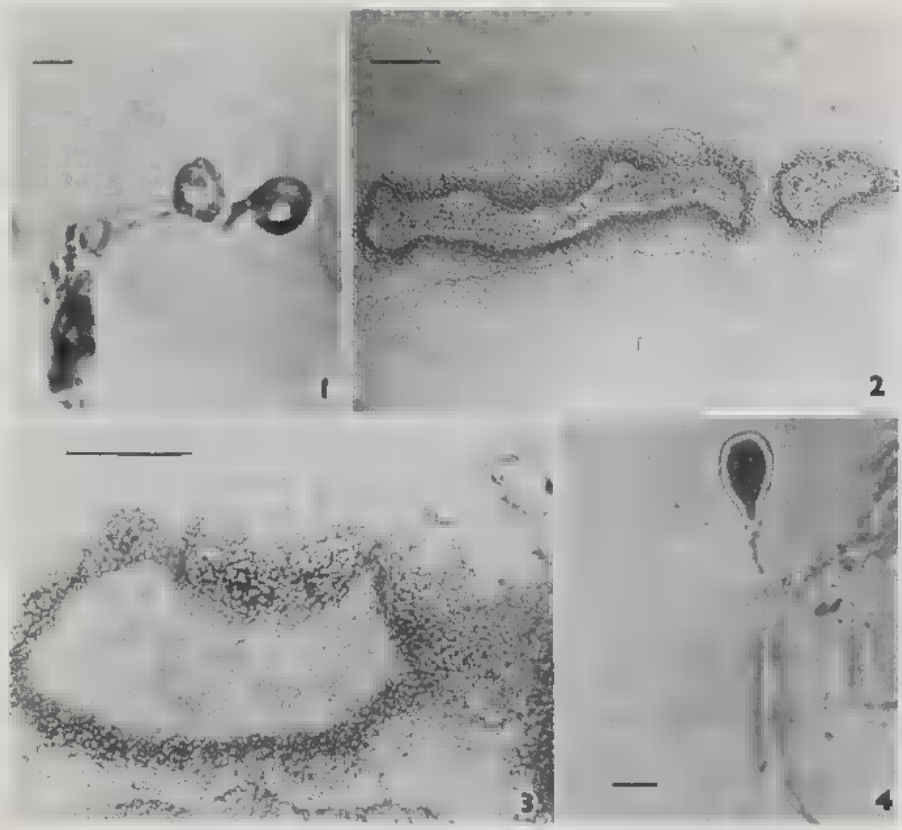
Hyphae hyaline, closely to remotely septate, up to 15μm wide (fig. 5, j, k, m, n, q). Hyphal fusions often observed (fig. 5, l, p). Raquette hyphae present (fig. 5, o). «Ascocarp» is an ascostroma. Ascostroma prosenchymatous, formed by the aggregation of masses of narrow hyphae. Ascogonia curled, numerous scattered within the stroma. Mature ascocarps (= ascostroma) variously shaped mostly spherical to elongate (fig. 4, a; Pl. I, fig. 3), single or clustered or confluent, light brown in colour, variable in size, up to 1.3 mm x 250μm, with an outer covering composed of pseudoparenchymatous tissue of irregularly arranged cells. Asci formed from croziers in groups, irregularly disposed, subglobose to ovate, 8-spored, 8-13.5 x 7-9.5μm (fig. 3, c-e). Ascospores hyaline, elliptical, slightly rough, 4.5-6.5 x 3.5-5μm (fig. 4, e, f). Gangliar conidia present, pro-



Pl. I. - *Thermoascus aurantiacus* - (Échelle: 10μm). Fig. 1: ascogonial curls (teased out). Fig. 2 & 3: Stages in the development of ascostroma. Note cavity in each ascostroma within which asci develop. Note clear pseudoparenchymatous nature of the tissue surrounding the cavity in fig. 3. Fig. 4: a mature gangliar conidium.

duced terminally on long or short hyphal branches, single or in clusters of 2 or 3, clavate or almost subglobose, smooth, thick-walled, up to 20 x 8 μm.

DEVELOPMENT OF THE TELIOMORPH

The teliomorph appears 2 or 3 days after the fungus is inoculated on YpSs agar medium and, being thermophilic, the culture needs to be incubated at a temperature of 38-40°C. Initially, small cushion like hyphal masses (prosenchymatous stroma) are formed by the aggregation of groups of profusely branched interwoven narrow hyphae produced by the ordinary vegetative mycelium (fig. 1, a). During the process, a marked change in the appearance of the culture is observed - the white to light grey-buff mucoraceous mycelial colony becoming granular and almost pale yellow.

A few deeply staining, smooth hyphal extensions now arise from some of the hyphal branches within each of these hyphal masses and become curled and septate; these are the ascogonia (fig. 1, b-i; Pl. I, fig. 1), and the hyphal mass with the ascogonia forms the rudiment of an ascostroma (fig. 1, a). The number of the turns in each of these ascogonial curls may vary from 1-3, and these curls are arranged somewhat loosely. Septation of these ascogonial curls may occur at an early stage in the development. No antheridium was seen. Also, no evidence of fusion between any of the ascogonial curls and the neighbouring hyphae was obtained. However, in certain cases, the ascogonial curls were found to be twisted around hyphal branches in the stroma.

With further development, the hyphae in the peripheral part of the prosenchymatous stroma become densely branched and knotted, tightly arranged and finally pseudoparenchymatous, forming an outer covering (fig. 2, a; Pl. I, fig. 2).

The next stage in development is marked by the formation of croziers and asci. As the ascogonia develop further, protuberances arise from some of the cells of the ascogonial curls; these protuberances elongate and become bent to form hook-like structures, the croziers (fig. 2, b-e). Formation of two septa divides each crozier into three cells; the basal, the penultimate and the tip cells (fig. 2, f). In some cases, before septation occurs, each hook produces one or two short extensions which may in turn develop into croziers (fig. 2, g, h). These croziers also become three-celled by septation. Invariably, the penultimate cell of each of these croziers proliferates to produce secondary croziers and a repetition of this process may lead to the formation of tertiary and quaternary croziers, etc. (fig. 2, i). After some time further croziers are not formed and the penultimate cells of the croziers already formed enlarge and become asci (fig. 2, o).

Sometimes, the protuberances produced on the ascogonial curls may not produce croziers as described above. Instead, loop-like structures may be formed (fig. 2, j, k) presumably by fusion of protuberances formed from adjacent cells or one protuberance from one cell of the ascogonial coil becoming bent and

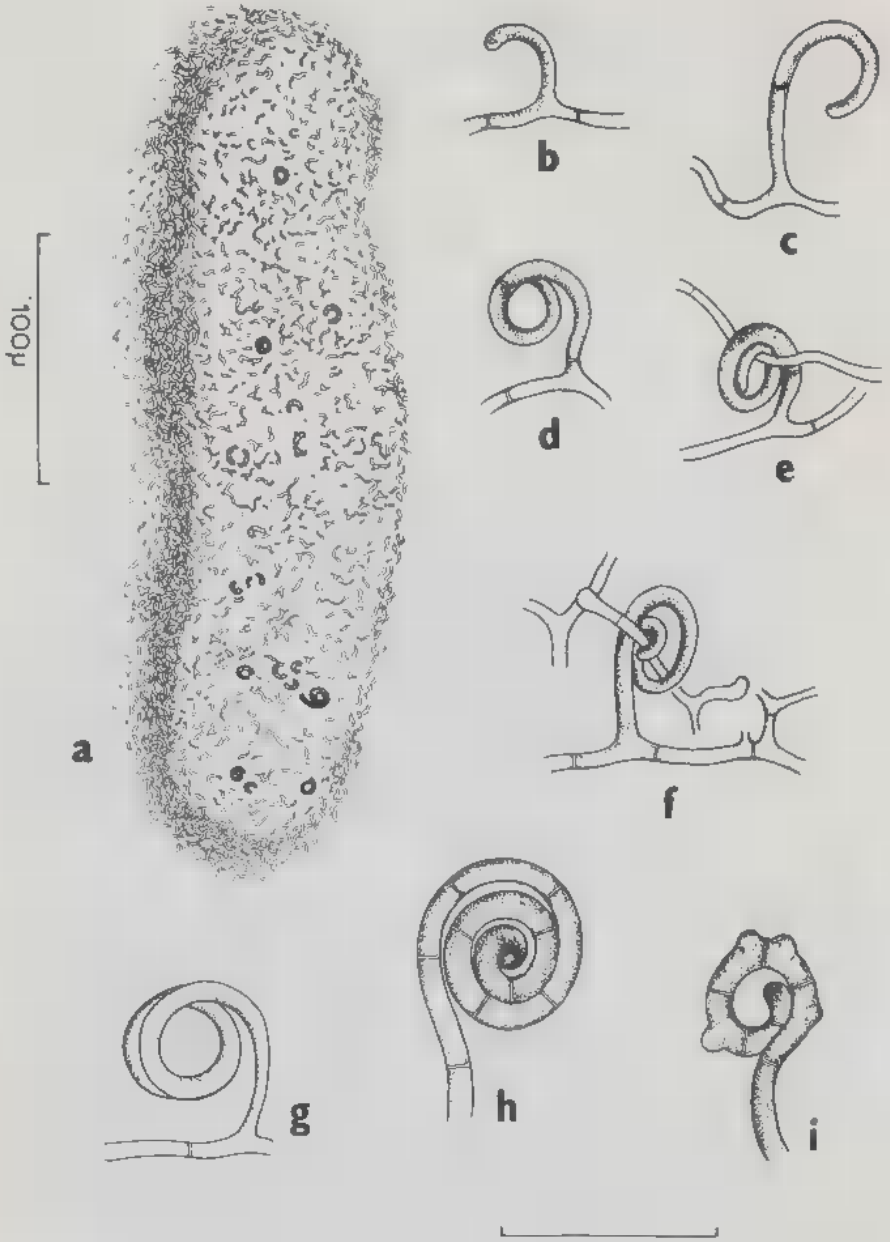
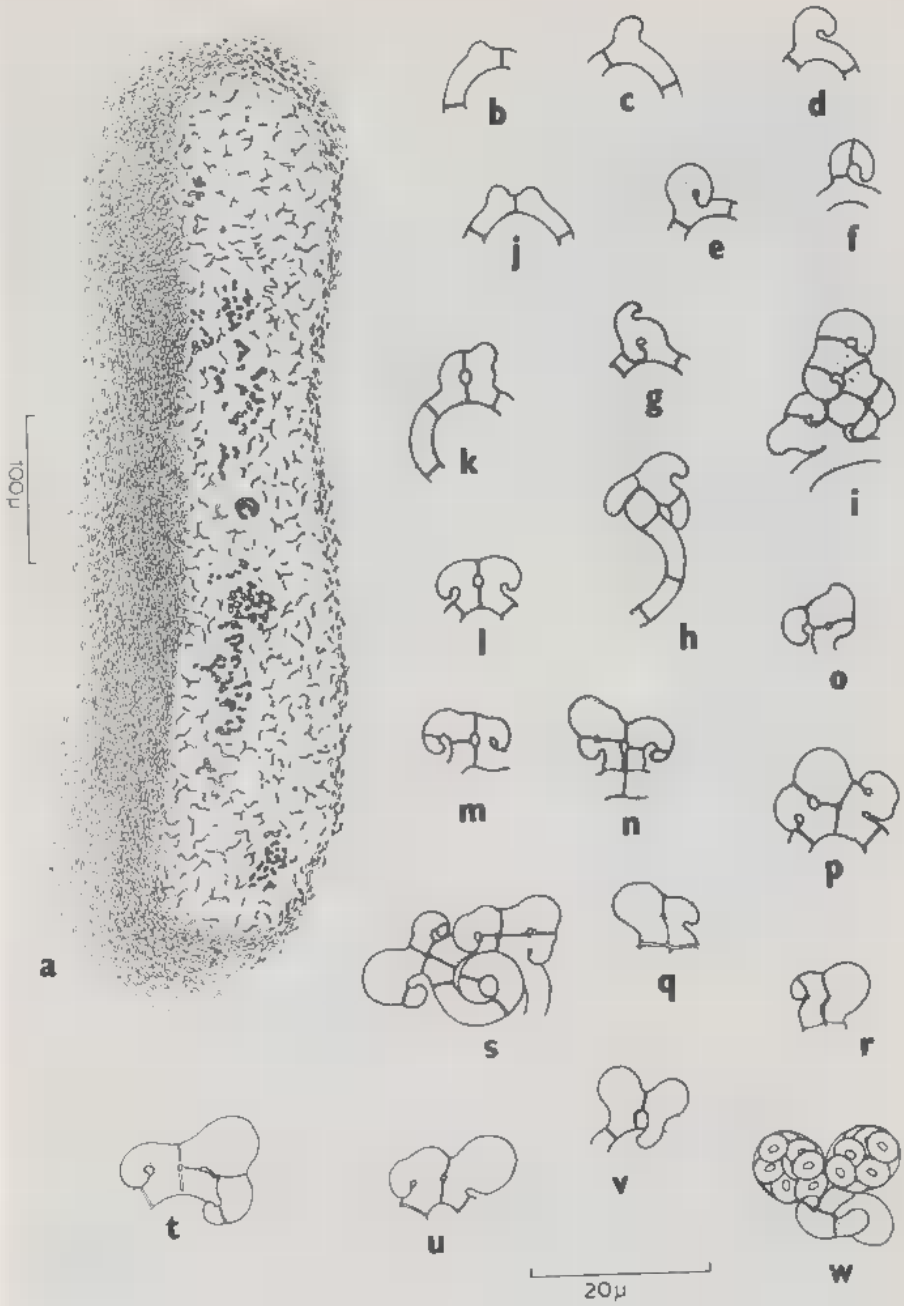


Fig. 1. - *Thermoascus aurantiacus* — a, showing a stroma containing several ascogonial curls (section); b-g, development of ascogonial curls - note in fig. a and f, vegetative hypha entangled within the curl; h, ■ septate ascogonial curl; i, initiation of croziers from cells of an ascogonial curl.



g. 2. - *Thermoascus aurantiacus* — a, stroma at a later stage of development - note that the basipetal region of stroma is more compact than before (compare fig. 1, a) - note also loosely woven stromal hyphae and ascogonial curls and croziers (section); b-f, development of croziers; g-i, production of secondary, tertiary and quaternary croziers; j-m, another pattern of crozier formation; n-w, development of asci and ascospores.

establishing connection with the adjacent cell. A septum now cuts the loop into two halves; each half develops a crozier in the usual way (fig. 2, k-m). The penultimate cell of each crozier enlarges and becomes an ascus. The tip cell of each crozier now becomes bent down and fuses either with the basal cell of the crozier (fig. 2, n) or the neighbouring ascogonial cell (fig. 2, p) and the process may be repeated so that several groups of croziers and asci are seen to develop from each ascogonial curl (fig. 2, q-w).

Finally eight ascospores become differentiated within each ascus. The asci lie scattered irregularly throughout the central tissue of the stroma (fig. 3, a, b).

The central system of narrow hyphae from which the ascogonial curls originally developed are seen to remain in the central part of the stroma even after the asci develop and mature (fig. 3, a, b), though they slowly get disintegrated in the final stage of maturation of the ascocarp.

The asci deliquesce and free the ascospores so that the ascospores from the numerous asci now fill the central cavity of the stroma (fig. 4, a; Pl. 1, fig. 3). At this stage in the development of the stroma, the fungus colony becomes almost brown in colour. No «ostiole» was observed.

Sometimes, as development proceeds, the individual stromata enlarge and get pressed against each other. Their wall layers get compressed and, by the dissolution of intervening wall layers, they merge into each other so that an apparently large stroma may be formed by the coalescence of more than one stroma. Such large stromata are frequently observed in somewhat aged cultures.

A section of mature stroma shows a large central cavity with ascospores lying free within and surrounded by an irregular tissue of polyhedral cells of somewhat two distinct zones - an inner deeply staining zone of comparatively smaller cells, and an outer somewhat hyaline not so deeply staining zone of larger cells; remnants of loose narrow hyphal branches are seen to project from the outer layer of this pseudoparenchymatous tissue.

GERMINATION OF ASCOSPORES (Fig. 4, g-o)

The ascospores germinate very rapidly. Germination takes place within 6 hours of incubation on dialysis tubings placed on YpSs agar medium at a temperature of 38-40°C. During germination, a small pore is formed on one side of the ascospore, mostly on the ventral side. Through this pore a protrusion emerges from within and assumes a vesicular appearance; this vesicular aspect is retained in further development. One or more extensions (germ tubes) develop from the vesicular part and grow. These branches become septate and produce the vegetative mycelium.

In certain cases, especially when ascospore germination was studied directly on media (i. e., not on dialysis tubing), the ascospore swells considerably, and one or more germ tubes emerge directly from within the ascospore. In

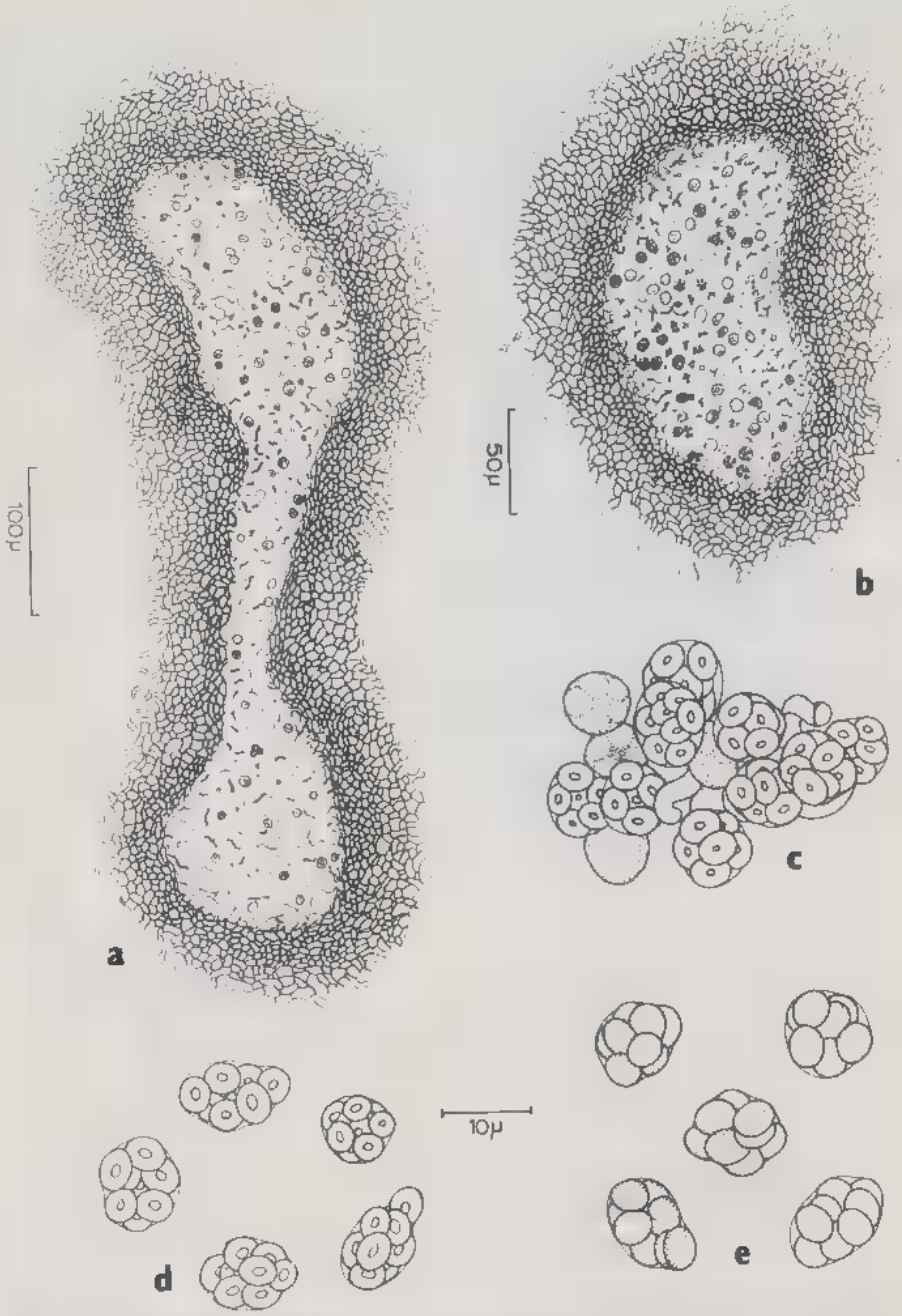


Fig. 3. - *Thermoascus aurantiacus* - a, b, section of ascostroma with central cavity and well developed pseudoparenchymatous tissue all round - note the presence of vegetative narrow hyphae and asci in the cavity; c, a group of asci; d, asci (? young); e, mature asci with ascospores.

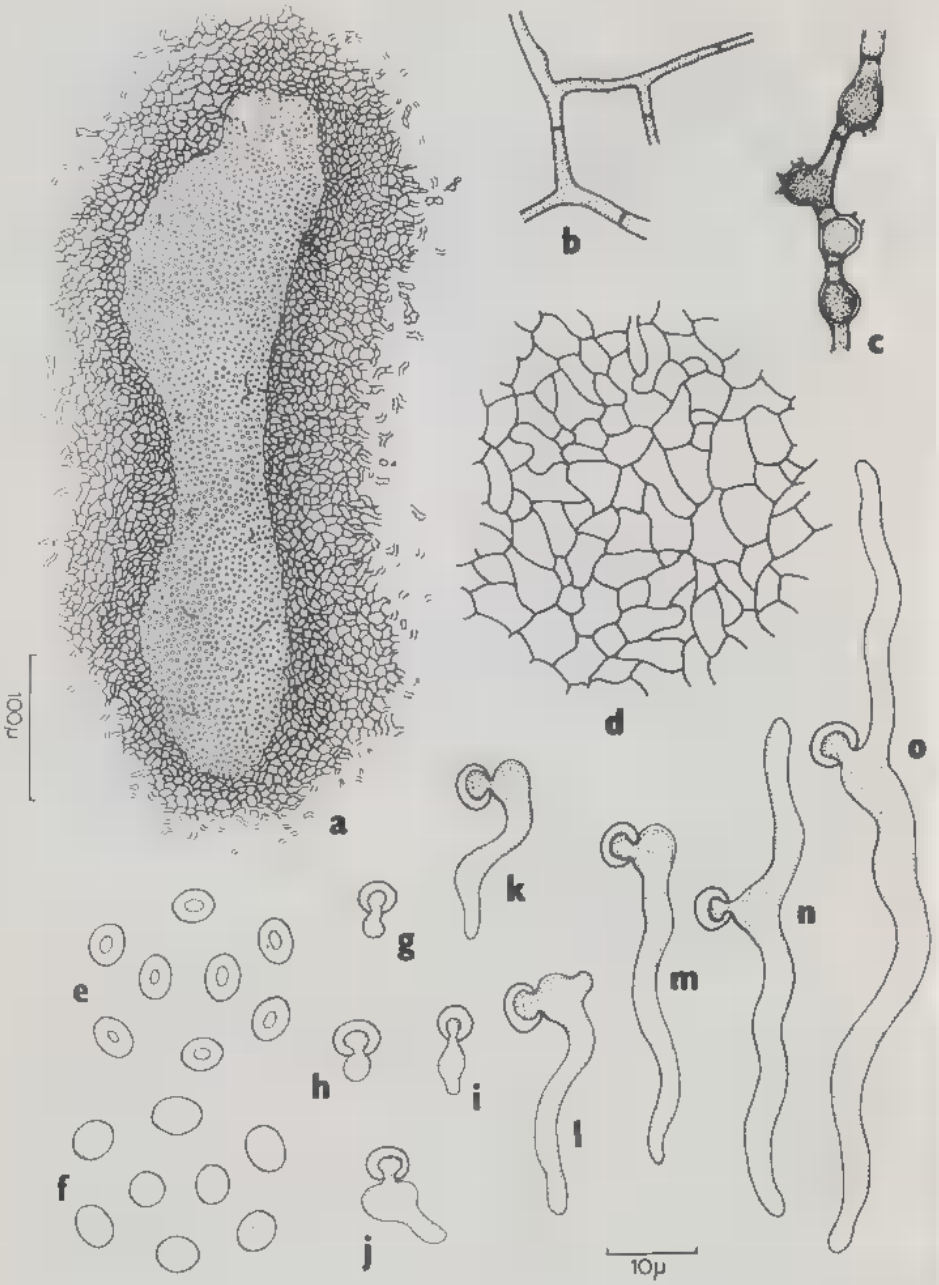


Fig. 4. - *Thermoascus aurantiacus* – a, mature ascostroma with ascospores within the stromal cavity (section); b, stromatic hyphae, young stage; c, stromatic hyphae, older stage; d, surface view of a part of ascostroma; e, mature ascospores; f, ascospores (? young); g-o, germination of ascospores.

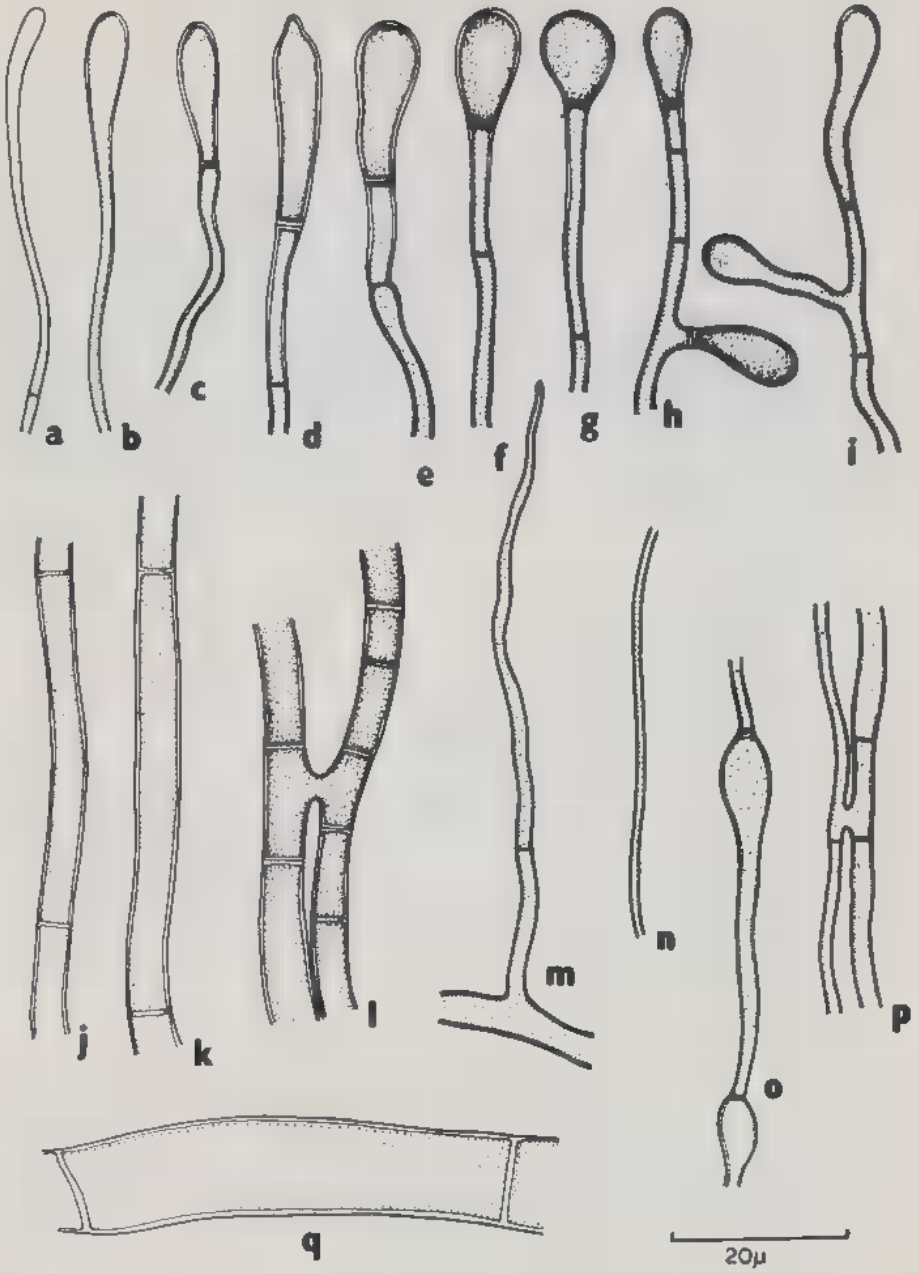


Fig. 5. - *Thermoascus aurantiacus* — a-i, development of gangliar conidia; j-q, variation in the size of hyphae - note hyphal fusion in fig. l and p and raquette hypha in fig. o.

such case, the vesicular structure is not visible outside the ascospore during germination.

DEVELOPMENT OF THE ANAMORPH

The present fungus was not found to produce any phialidic state. However, the production of gangliar conidia was frequently observed in cultures grown on YpSs agar medium.

During the formation of gangliar conidia, the terminal portion of an ordinary vegetative hypha swells (fig. 5, a, b). The swelling increases in size as it develops further and is then cut off by a septum separating the swelling from the subtending hyphae (fig. 5, c-g). Due to further development the wall of the swelling becomes thick, and finally this terminal swollen part becomes transformed into a gangliar conidium (Pl. I, fig. 4). Later, from any point on the conidiophore one or two lateral branches arise; the terminal portion of each of these lateral branches may also swell and become gangliar conidia (fig. 5, h, i).

TAXONOMY

The genus *Thermoascus* Miehe (MIEHE, 1907) is based on *T. aurantiacus* Miehe. Unfortunately, no type material seems to exist and the present study is based on IMI 67936.

The unique features of this fungus are as follows. The ascocarp is an ascostroma. To begin with, the ascostroma is prosenchymatous and of variable extent (fig. 1, a). Several ascogonia develop within an ascostroma. A mature ascocarp has a distinct and conspicuous envelope of pseudoparenchymatous cells of variable thickness, usually 8-12 cells thick (fig. 4, a). This is what has been usually referred to in descriptions as the peridium. However, from the present developmental study we are inclined to believe that this pseudoparenchymatous tissue represents the finale in development of the ascostroma so that we now have in place of prosenchymatous tissue a pseudoparenchymatous one. If this interpretation is correct, then the ascocarp in this fungus lacks a peridium sensu stricto.

No phialide state developed in culture such as has been claimed by COONEY and EMERSON (1964). However, conidia of gangliar type were found. It is noteworthy that MIEHE also found no phialide state associated with this fungus.

Thermoascus seems to be a good genus and must be retained. It is quite distinct from the other genera studied by the authors. As interpreted here, it is an ascostromatic form in which the ascocarp is without a peridium and develops in a cavity in an ascostroma.

We are grateful to the Director, C.M.I., for the culture of *Thermoascus aurantiacus* on which the present study is based.

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